Original Research Article

Effect of botanicalextracts and essential oils on *Alternariabrassicae* of mustard(*Brassica juncea*L.)

ABSTRACT

Mustard is an important oilseed crop in India. Alternaria leaf blight caused by *Alternariabrassicae*is an economically important disease of oilseed brassicas as it reduces the quality and quantity of the seeds. Botanical extracts and essential oils were tested on *Alternariabrassicae* at different concentrations to evaluate the effect *in vitro* by poison food technique in Completely Randomized Design (CRD). The studywas done at the Department of Plant Pathology laboratory, Sam Higginbottom University of Agriculture, Technology And Sciences during 2021-2022.The results revealed that all the botanical extracts and essential oils tested significantly inhibited the mycelial growth of *Alternariabrassicae*. Among the botanical extracts, the highest mycelial growth inhibition was found in garlic bulb extract @ 15% (93.11%), followed by garlic bulb extract @ 10% (87.33%). Among essential oilsthe maximum mycelial growth inhibition per cent was observed in eucalyptus oil @ 3% (95.77%),followed by eucalyptus oil @ 2% (88.22%). The higher concentration of all the botanical extracts and essential oils shows significantly higher per cent mycelial growth inhibition as compared to their lower levels.

Keywords: Alternariabrassicae, botanical extracts, essential oils, in vitro, mustard

1. INTRODUCTION

Indian mustard (*Brassica juncea* L.) is an important oilseed crop grown both in tropical and sub-tropical regions of the world. Alternaria blight is widely distributed disease in all mustard cultivated areas[1].The symptoms of *A. brassicae* appear on leaves, stem, adult plants and also in siliquae during ripening stage. Dark spotsappears on leaves and siliquae which adversely affect on seed production and quality of mustard [2]. It cause high yield loss, 15-71% in productivity with 14-36% decrease in oil content [3].Natural plant extracts modulate plant growth and are involved in plant defense responses, including limiting pathogen development [4]. The action of natural compounds such as terpenoids, phenolics, and alkaloids are not specific, and their effects on pathogens are versatile [5].Essential oils constitute an important source of biologically active compounds, including antimicrobial, insecticidal, fungicidal, nematicidal, herbicidal, antioxidant and anti-inflammatory properties[6].

Comment [M1]: Aim of the study is missing

2. MATERIALS AND METHODS

The experiment was conducted in theDepartment of Plant Pathology Laboratory, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj, U.P. India during 2022. Laboratory experiment was carried out to find out the inhibitory effect of botanical extracts and essential oil on the mycelial growth of *Alternariabrassicae* in Completely Randomized Design (CRD) with five replications in each treatment.

2.1 Isolation and maintenance of pure culture

Pathogenic *Alternariabrassicae* was isolated from infected leaf of mustard collected from Central Research Field of SHUATS. Infected portions with concentric rings were cut out from leaves for microscopic examination to check the presence of pathogenic fungus. After confirming the presence of *Alternariabrassicae*, leaves were cut into small pieces (1-1.5cm) with sterile blade. These pieces were disinfected with 0.5% sodium hypochlorite (NaOCl) solution for two minutes followed by three washings with distilled water and excessive moisture was removed using sterile blotter paper. The surface sterilized leaf pieces were aseptically transferred into petri dishes containing PDA medium using sterilized forceps and incubated at 25±2 °C for 7 days.

Comment [M2]: Expand

Microscopic observation confirmed the presence of *Alternariabrassicae*. Pathogen pure culture was performed on PDA slants and petri plates under aseptic condition by single spore technique. The plates were incubated at 25°C for 1 week until full growth obtained and pathogen sub culture was done aseptically for further study.

2.2 Preparation of plant extracts

Matured leaves were collected and sterilized with distilled water, the leaves were homogenized in a pre-chilled pestle and mortar using chilled and sterilized distilled water. Aqueous extract of this botanical (1% w/v) was prepared by mixing 100g fresh leaves of plant with 100 ml of sterile distilled water and crushing in warring blender. The extract was filtered through four layers of moisture muslin cloth. The filtrate thus obtained was considered as 100% concentration. Aqueous extracts (5, 10 and 15%) were prepared according to the treatments and mixed with 100ml PDA respectively in separate 250ml conical flask. The media in conical flask was sterilized in an autoclave at temperature of 121°C for 20 minutes.

Comment [M3]: Name the plant

Comment [M4]: Mention the plant

2.3 Poison food technique:

2.3.1 Evaluation of plant extracts on the colony growth of test fungus

The procedure of the experiment was done according to [7]. An appropriate quantity of plant extracts at desired concentrations were mixed thoroughly, autoclaved and cooled (40°C) PDA medium in conical flasks (250ml cap) to obtain desired concentrations (5, 10 and 15%). Sterilized and cooled PDA medium amended with plant extracts was then poured (15 to 20 ml/plate) into sterile glass petri plates (90mm) and allowed to solidify at room temperature. The plant extracts and its respective concentrations were replicated five times. The plates containing PDA without any plant extract were

Comment [M5]: Which plant?

maintained as control. Upon solidification of PDA, the treated and control plates were aseptically inoculated by placing a 5mm mycelial disc in the centre obtained from a week old actively growing pure culture of *A. brassicae*. All these plates were then incubated at 25±1°C temperature till the treated control plates were fully covered with mycelial growth of the test fungus. Per cent inhibition of growth (mm) of *A. brassicae* in petri plate was recorded at 7th day. The radial growth of mycelium of each plate was measured by taking average of the two diameters taken at right angles for each colony.

2.3.2 Evaluation of essential oils on the colony growth of test fungus

The evaluation of antifungal activity of the essential oils using the poisoned food technique was done according to [7], required amount of the stock solutions from essential oils(1ml, 2ml and 3ml) mixed with Tween 20 were incorporated to enhance oil solubility into the sterile PDA medium and then poured in the petri dishes (90 mm) at 45°C and the control plates were carried out only in PDA without adding the oil. Essential oils and its respective concentrations were replicated five times.

Comment [M6]: Which essential oil?

2.3.3 Evaluation of fungicide on the colony growth of test fungus

Mancozeb (Dithane M-45) @ 0.2% was dissolved in 100 ml of sterilized molten PDA prior to inoculation of *Alternariabrassicae*. PDA plates without chemical but inoculated with *Alternariabrassicae*were served as control. Five replications were maintained for all the treatments and plates were incubated in BOD incubator at a temperature of 25±1°C. The colony diameter of the fungus was measured on 7th day of incubation and compared with the colony growth of the fungus in control. The experiment was conducted in Completely Randomized Design (CRD) with five replications in each treatment.

The inhibition percentage was calculated using the formula:

$$I = \frac{C - T}{C} \times 100$$

where,

I = Per cent inhibition

C = Growth (mm) of test fungus in untreated control plates (average of both diagonals),

T = Growth (mm) of test fungus in treated plates (average of both diagonals).

3. RESULTS AND DISCUSSION

Three botanical extracts,*viz*., garlic bulb extract, neem leaf extract and *Lantana camara* leaf extract @ 5%, 10%, 15% concentrations and two essential oils,*viz*., eucalyptus oil and neem oil @ 1%, 2%, 3% concentrations were evaluated on *Alternariabrassicaeinvitro* and the results are presented on table 1, figure 1 and plate I, II, III.

3.1 Effect of botanical extracts and essential oils on the mycelial growth of *Alternariabrassicae*

The data presented in table 1, depicted in figure 1 and plate I reveal that all the treatments significantly inhibited the radial growth of *Alternariabrassicae* when compared to untreated control. Among plant extracts @ 5% concentration garlic bulb extract showed maximum inhibition of radial growth (74.22%), followed by neem leaf extract (3.11%), *Lantana camara* leaf extract (4.66%). Among essential oils @ 1% concentration, eucalyptus oil showed maximum inhibition of radial growth (77.11%), followed by neem oil (22.22%) as compared to mancozeb (treated check) @ 0.2% (100%) and untreated control (0%).

Comment [M7]: Name the control

The data presented in table 1, depicted in figure 1 and plate II reveal that all the treatments significantly inhibited the radial growth of *Alternariabrassicae* when compared to untreated control. Among plant extracts @ 10% concentration, garlic bulb extract showed maximum inhibition of radial growth (87.33%), followed by neem leaf extract (46.22%), *Lantana camara* leaf extract (10.66%). Among essential oils @ 2% concentration, eucalyptus oil showed maximum inhibition of radial growth (88.22%), followed by neem oil (31.33%) as compared to mancozeb (treated check) @ 0.2% (100%) and untreated control (0%).

The data presented in table 1, depicted in figure 1 and plate III reveal that all the treatments significantly inhibited the radial growth of *Alternariabrassicae* when compared to untreated control. Among plant extracts @ 15% concentration, garlic bulb extract showed maximum inhibition of radial growth (93.11%), followed by neem leaf extract (71.55%), *Lantana camara* leaf extract (17.11%). Among essential oils @ 3% concentration, eucalyptus oil showed maximum inhibition of radial growth (95.77%), followed by neem oil (37.77%) as compared to mancozeb (treated check) @ 0.2% (100%) and untreated control (0%).

All the treatments are statistically significant over control. Among the treatments, eucalyptus oil @ 2% and garlic bulb extract @ 10%, *Lantana camara* leaf extract and neem leaf extract @ 5% were statistically at par.

From the study, it was evident that mycelial growth inhibition of the pathogen increased linearly with an increase in concentration of botanical extracts and essential oils. Among the botanical extracts, the highest mycelial growth inhibition was found in garlic bulb extract @ 15% (93.11%), followed by garlic bulb extract @ 10% (87.33%). Among essential oils, the maximum mycelial growth inhibition per cent was observed in eucalyptus oil @ 3% (95.77%), followed by eucalyptus oil @ 2% (88.22%).

Concurrent with present findings, [8] have reported maximum inhibition of mycelial growth with different concentration of garlic bulb extracts. The effectiveness of garlic bulb extract in inhibition of mycelial growth of the pathogen might be due to the presence of antimicrobial properties like diallyl-disulfide and diallyl-trisulfide in *Allium sativum*[9]. Similar results were earlier reported by [10] and [11]. [12] reported maximum inhibition of mycelial growth of eucalyptus oil.Probable reasons may be due to the main constituents of eucalyptus oil like isoborneol, linalool, α-terpineol, 1,8-cineole, α-

pinene and camphene which have known antimicrobial properties [13]. Similar results were also reported by [14] and [15].

Table 1 Effect ofbotanical extracts and essential oils on the mycelial growth of *Alternariabrassicae*

Tr.No	Treatments	Concentrations	Radial growth of the pathogen(mm)	Per cent inhibition
T_0	Control (untreated check)	-	90	0
T_1	Garlic bulb extract	5%	23.20	74.22
		10%	11.40	87.33
		15%	6.20	93.11
T_2	Neem leaf extract	5%	87.20	3.11
		10%	48.40	46.22
		15%	25.60	71.55
T_3	*Lantana camara*leaf extract	5%	85.80	4.66
		10%	80.40	10.66
		15%	74.60	17.11
T_4	Eucalyptus oil	1%	20.60	77.11
		2%	10.60	88.22
		3%	3.80	95.77
T_5	Neem oil	1%	70.00	22.22
		2%	61.80	31.33
		3%	56.00	37.77
T_6	Mancozeb (treated check)	0.2%	0	100
		S.Em (±)	**C.D. (*P*=0.05)**	
	Treatments	0.4	1.07	
	Concentrations	0.24	0.70	
	T×C	0.73	1.85	

*****Averageoffivereplications.

Valuesinthesamecolumnfollowedwithsimilaralphabetarenon-significanttoeach otherat(p=0.05).

Comment [M8]: Follow the same format (provide common name or botanical)

Comment [M9]: No details about statistical analysis in materials and methods

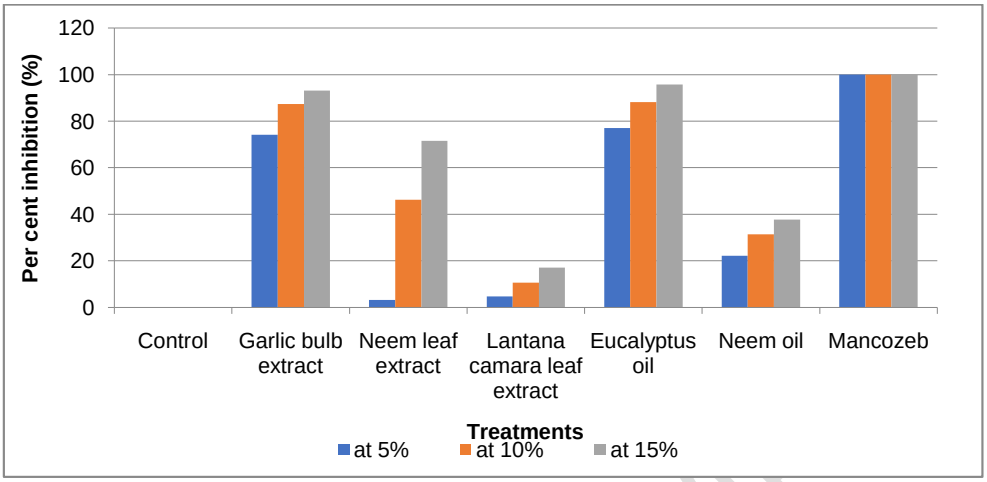

Figure 1 Effect of botanical extracts and essential oils on the mycelial growth inhibition per cent of *Alternariabrassicae*

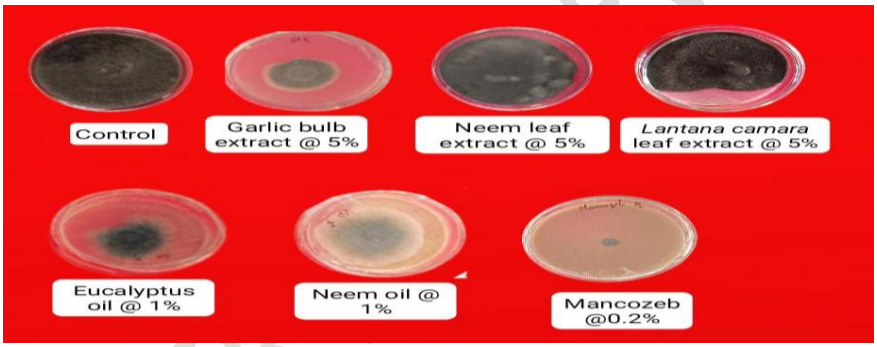

Plate I. Effect of botanical extracts (5%) and essential oils (1%) on the radial growth of *Alternariabrassicae*

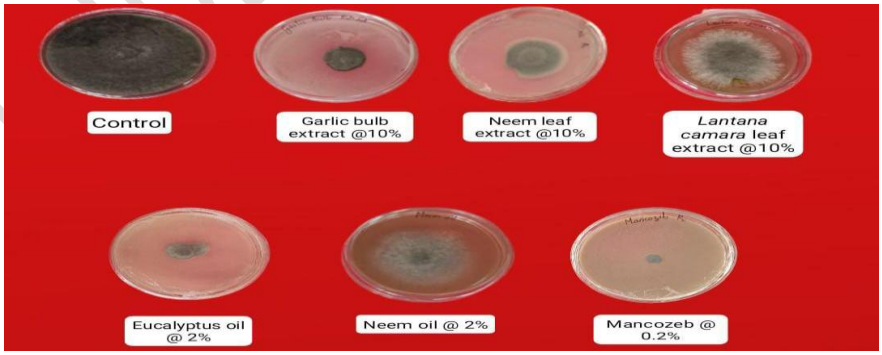

Plate II. Effect of botanical extracts (10%) and essential oils (2%) on the radial growth of *Alternariabrassicae*

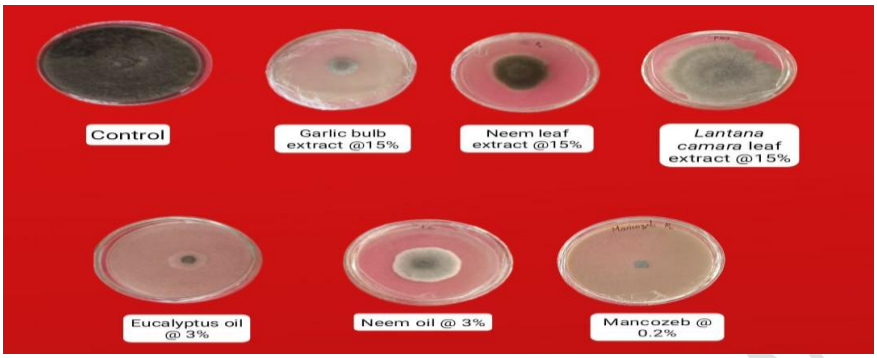

Plate III. Effect of botanical extracts (15%) and essential oils (3%) on the radial growth of *Alternariabrassicae*

CONCLUSION

It was concluded that both eucalyptus oil as well as garlic bulb extract effectively inhibited the growth of *Alternariabrassicae* in mustard. Botanical extracts and essential oils are environmently non pollutants, non-phytotoxic, cost effective and having antifungal properties which inhibited the pathogen growth,thus can be used as a substitute for synthetic chemicals. For substantiation of current result, more trials should be conducted in future for further recommendations.

Comment [M10]: Only with this study, how can this be concluded?

REFERENCES

1. Sohi GS, Singh A, Singh B. Symptoms and management of alternaria blight of Indian mustard (*Brassica juncea*). Journal of Plant Development Sciences. 2020;12(3):165-170.
2. Kumar D, Maurya N, Bharti YK, Kumar A, Kumar K, Srivastava K et al. Alternaria blight of oilseed Brassicas: A comprehensive review. African Journal of Microbiology. 2014;8(30): 2816-2829.
3. Meena PD, Awasthi RP, Chattopadhyay C, Kolte SJ, Kumar A. Alternaria blight: A chronic disease in rapeseed mustard. Journal of Oilseed Brassica. 2010;1(1):1-11.
4. Jamiołkowska A. Natural compounds as elicitors of plant resistance against diseases and new. Bio-control strateges. Agronomy. 2020;10(2):173.
5. Righini H, Francioso O, Di Foggia M, Quintana AM, Roberti R. Assessing the potential of the terrestrial cyanobacterium *Anabaena minutissima* for controlling *Botrytis cinerea* on tomato fruits. Horticulturae. 2021;7(8):210-216.
6. Bassole IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012; 17:3989–4006.

7. Nene YL, Thapliyal RN. Evaluation of fungicides in fungicides for plant disease control. 3rd ed. Oxford and ibh Publishing Company Private Limited of New Delhi; 1993.
8. Singh, A. K. and Tiwari, A. K. (2007). Effect of plant extract against Alternariabrassicae the incitant of leaf spot of crucifers. National Symposium on advancing frontiers of plant disease management, Department of Botany, KNIPPS, Sultanpur. 67.
9. Kumar SP, Mishra MK, Mishra PR. In vitro efficacy of botanicals and biocontrol agents against early leaf blight in tomato. International Journal of Current Microbiology and Applied Sciences. 2018;7(12):1340 -1345.
10. Singh M, Singh HK. *In vitro* studies of plant extracts against *Alternariabrassicae* (Berk.) Sacc. of Indian mustard [*Brassica juncea* (L.) Czern. &Coss]. Journal of AgriSearch. 2016;3(1):32-33.
11. Chakma T, Devi S, Sinha B, Chakrapani, K, Gopi N, Singh M, Devi R, SinghKH. In vitro studies on efficacy of plant extracts against *Alternariasolani* causing early blight of Potato. The Pharma Innovation Journal. 2023;12(5):04-07.
12. Yadav JK, Singh HK, Singh SK, Kavita, Singh S. Efficacy of plant extracts against *Alternariabrassicae* under in vitro condition. Journal of Pharmacognosy and Phytochemistry. 2019;8(1):528-532.
13. Park SN, Lim YK, Freire MO, Cho E, Jin D, Kook JK. Antimicrobial effect of linalool and α-terpineol against period on topathic and cariogenic bacteria. Anaerobe. 2012;18(3):369-372.
14. Singh HK, Singh RB, Shakywar RC. Alternaria blight of rapeseed-mustard and its management. Plant Disease Management for Food Security LAP LAMBERT Academic Publishing GmbH and Co. K. G. Heinrich- Boocking-str. 6-8, 66121, Saarbrucken, Germany; 2013.
15. Rahmatzai N, Zaitoun A, Madkour M, Ahmady A, Zainullah H, Mousa M. In vitro and in vivo antifungal activity of botanical oils against *Alternariasolani* causing early blight of tomato. International Journal of Biosciences. 2017;10(1):91-99.